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- 1 1. A nucleic acid encoding a hybrid polypeptide comprising a signal sequence  
2 and three segments, wherein the three segments are either contiguous or are separated by  
3 a spacer amino acid or spacer peptide:  
4 (a) the first segment having the amino acid sequence of a first portion of a  
5 naturally occurring tumor antigen or naturally occurring protein of a pathogenic agent,  
6 the first segment being at least eleven amino acids in length and comprising two epitopes;  
7 (b) the second segment having the amino acid sequence of a second portion of a  
8 naturally occurring tumor antigen or naturally occurring protein of a pathogenic agent,  
9 the second segment being at least eleven amino acids in length and comprising two  
10 epitopes different from the epitopes of (a); and  
11 (c) the third segment having the amino acid sequence of a third portion of a  
12 naturally occurring tumor antigen or naturally occurring protein of a pathogenic agent,  
13 the third segment being at least eleven amino acids in length and comprising two epitopes  
14 different from the epitopes of (a) and (b),  
15 provided that either  
16 (i) the first, second and third portions are non-contiguous portions of the same naturally  
17 occurring protein, and the sum of all three portions constitutes less than 70% of the  
18 sequence of the naturally occurring protein; or  
19 (ii) the first, second and third portions are portions of three different naturally occurring  
20 tumor antigens or naturally occurring proteins of one or more pathogenic agents.

- 1 2. The nucleic acid of claim 1, wherein at least one of the segments comprises  
2 three epitopes.

- 1 3. The nucleic acid of claim 1, wherein at least one of the segments comprises  
2 four epitopes.

- 1 4. The nucleic acid of claim 1, wherein at least three of the epitopes are MHC  
2 class I-binding epitopes.

- 1 Sub 2 5. The nucleic acid of claim 1, further comprising

2 (d) a fourth segment which has the amino acid sequence of a fourth portion of a  
3 naturally occurring tumor antigen or naturally occurring protein of a pathogenic agent,  
4 the fourth segment being at least eleven amino acids in length and comprising two  
5 epitopes different from the epitopes of (a), (b) and (c).

1 6. The nucleic acid of claim 5, wherein the fourth segment has the amino acid  
2 sequence of a portion of a naturally occurring protein that is different from the naturally  
3 occurring protein of (a).

1 7. The nucleic acid of claim 1, wherein at least one of the segments is less than  
2 15 amino acids in length.

1 8. The nucleic acid of claim 1, wherein at least one of the segments has the  
2 sequence of a portion of a human papilloma virus (HPV) protein.

1 9. The nucleic acid of claim 1, wherein each of the naturally occurring proteins is  
2 an HPV protein.

1 10. The nucleic acid of claim 1, wherein at least two of the segments are  
2 contiguous.

1 11. The nucleic acid of claim 1, wherein the three segments are contiguous.

1 12. The nucleic acid of claim 1, wherein the first and second segments are  
2 separated by a spacer amino acid or a spacer peptide and the second and third segments  
3 are separated by a spacer amino acid or a spacer peptide.

1 13. The nucleic acid of claim 1, wherein the first and second segments are  
2 separated by a spacer amino acid and the second and third segments are separated by a  
3 spacer amino acid.

1           14. The nucleic acid of claim 1, wherein the first and second segments are  
2 separated by a spacer amino acid which is alanine and the second and third segments are  
3 separated by a spacer amino acid which is alanine.

1           15. The nucleic acid of claim 12, wherein each of the naturally occurring proteins  
2 is an HPV protein.

1           16. The nucleic acid of claim 13, wherein each of the naturally occurring proteins  
2 is an HPV protein.

1           17. The nucleic acid of claim 14, wherein each of the naturally occurring proteins  
2 is an HPV protein.

1           18. The nucleic acid of claim 8, wherein the hybrid polypeptide comprises a first  
2 epitope from an HPV protein and a second epitope which does not overlap with the first  
3 epitope and which is from the same or a different HPV protein, wherein the first epitope  
4 binds to a first major histocompatibility complex (MHC) class I allotype and the second  
5 epitope binds to a second MHC class I allotype different from the first MHC class I  
6 allotype.

1           19. The nucleic acid of claim 18, wherein at least one of the portions is from an  
2 HPV E6 or HPV E7 protein.

1           20. The nucleic acid of claim 18, wherein at least one of the portions is from an  
2 HPV strain 16 protein or an HPV strain 18 protein.

1           21. The nucleic acid of claim 18, wherein at least one of the portions is from an  
2 HPV E6 or E7 protein of HPV strain 16 or 18 origin.

1           22. The nucleic acid of claim 18, wherein the first MHC class I allotype is  
2 selected from the group consisting of HLA-A1, HLA-A2, HLA-A3, HLA-A11, and  
3 HLA-A24.

1           23. The nucleic acid of claim 22, wherein the second MHC class I allotype is  
2 selected from the group consisting of HLA-A1, HLA-A2, HLA-A3, HLA-A11, and  
3 HLA-A24.

1           24. The nucleic acid of claim 18, wherein the hybrid polypeptide further  
2 comprises a third epitope from an HPV protein, wherein the third epitope binds to a third  
3 MHC class I allotype different from the first and second MHC class I allotypes.

1           25. The nucleic acid of claim 18, wherein the hybrid polypeptide comprises 10  
2 MHC class I allotype-binding epitopes from one or more HPV proteins.

1           26. The nucleic acid of claim 18, wherein the hybrid polypeptide comprises 40  
2 MHC class I allotype-binding epitopes from one or more HPV proteins.

1           27. The nucleic acid of claim 18, wherein the hybrid polypeptide comprises 60  
2 MHC class I allotype-binding epitopes from one or more HPV proteins.

1           28. The nucleic acid of claim 24, wherein the first epitope overlaps with the third  
2 epitope.

1           29. The nucleic acid of claim 1, wherein the signal sequence and the first  
2 segment are separated by a spacer amino acid or a spacer peptide.

1           30. The nucleic acid of claim 1, wherein the hybrid polypeptide comprises ten  
2 MHC class I-binding epitopes from one HPV protein.

1           31. The nucleic acid of claim 1, comprising

2 (a) a first plurality of HLA-binding epitopes from an HPV strain 16 E6 protein,  
3 and  
4 (b) a second plurality of HLA-binding epitopes from an HPV strain 16 E7 protein;  
5 wherein each of the HLA-binding epitopes binds to one or more allotypes selected  
6 from the group consisting of HLA-A1, HLA-A2, HLA-A3, HLA-A11, and HLA-A24.

1 32. The nucleic acid of claim 1, comprising

2 (a) a first plurality of HLA-binding epitopes from an HPV strain 18 E6 protein,  
3 and  
4 (b) a second plurality of HLA-binding epitopes from an HPV strain 18 E7 protein,  
5 wherein each of the HLA-binding epitopes binds to one or more allotypes selected  
6 from the group consisting of HLA-A1, HLA-A2, HLA-A3, HLA-A11, and HLA-A24.

1 33. The nucleic acid of claim 1, comprising

2 (a) a first plurality of HLA-binding epitopes from an HPV strain 16 E6 or E7  
3 protein, and  
4 (b) a second plurality of HLA-binding epitopes from an HPV strain 18 E6 or E7  
5 protein,  
6 wherein each of the HLA-binding epitopes binds to one or more allotypes selected  
7 from the group consisting of HLA-A1, HLA-A2, HLA-A3, HLA-A11, and HLA-A24.

1 34. The nucleic acid of claim 1, comprising

2 (a) a first plurality of HLA-binding epitopes from an HPV strain 16 E6 protein,  
3 (b) a second plurality of HLA-binding epitopes from an HPV strain 16 E7 protein,  
4 (c) a third plurality of HLA-binding epitopes from an HPV strain 18 E6 protein,  
5 and  
6 (b) a fourth plurality of HLA-binding epitopes from an HPV strain 18 E7 protein,  
7 wherein each of the HLA-binding epitopes binds to one or more allotypes selected  
8 from the group consisting of HLA-A1, HLA-A2, HLA-A3, HLA-A11, and HLA-A24.

1           35. The nucleic acid of claim 31, wherein each plurality of epitopes comprises at  
2 least five epitopes, each of which binds to one or more of the allotypes.

1           36. The nucleic acid of claim 31, wherein each plurality of epitopes comprises at  
2 least 15 epitopes, each of which binds to one or more of the allotypes.

1           37. A nucleic acid encoding a hybrid polypeptide comprising a signal sequence  
2 and three segments, wherein the three segments are either contiguous or are separated by  
3 a spacer amino acid or spacer peptide:

4           (a) the first segment having the amino acid sequence of a first portion of a  
5 naturally occurring HPV protein, the first segment being at least eleven amino acids in  
6 length and comprising two epitopes;

7           (b) the second segment having the amino acid sequence of a second portion of a  
8 naturally occurring HPV protein, the second segment being at least eleven amino acids in  
9 length and comprising two epitopes different from the epitopes of (a); and

10          (c) the third segment having the amino acid sequence of a third portion of a  
11 naturally occurring HPV protein, the third segment being at least eleven amino acids in  
12 length and comprising two epitopes different from the epitopes of (a) and (b),  
13 provided that either

14          (i) the first, second and third portions are non-contiguous portions of the same naturally  
15 occurring HPV protein, and the sum of all three portions constitutes less than 70% of the  
16 sequence of the naturally occurring protein; or

17          (ii) the first, second and third portions are portions of two or three different naturally  
18 occurring HPV proteins.

1           38. The nucleic acid of claim 37, wherein at least one of the segments comprises  
2 three epitopes.

1           39. The nucleic acid of claim 37, wherein at least one of the segments comprises  
2 five epitopes.

1        40. The nucleic acid of claim 37, wherein at least three of the epitopes are MHC  
2 class I-binding epitopes.

1        41. The nucleic acid of claim 37, further comprising  
2        (d) a fourth segment which has the amino acid sequence of a fourth portion of a  
3 naturally occurring HPV protein, the fourth segment being at least eleven amino acids in  
4 length and comprising two epitopes different from the epitopes of (a), (b) and (c).

1        42. A DNA encoding a hybrid polypeptide the sequence of which comprises at  
2 least one of the following segments of HPV strain 16 E6:

3        AMFQDPQERPRKLPQLCTEL (SEQ ID NO:64),  
4        LLRREVVYDFAFRDLCIVYRDGNPY (SEQ ID NO:65), and  
5        KISEYRHYCYSLYGTTLEQQYNK (SEQ ID NO:66),

6 and at least one of the following segments of HPV strain 16 E7:

7        TLHEYMLDLQPETTDLYSY (SEQ ID NO:67),  
8        QAEPDRAHYNIVTF (SEQ ID NO:68), and  
9        LLMGTLGIVCPICSQKP (SEQ ID NO:69),

10 provided that the hybrid polypeptide does not comprise a sequence identical to the  
11 sequence of either full length, intact E6 or full length, intact E7 protein from HPV  
12 strain 16.

1        43. The DNA of claim 42, wherein the hybrid polypeptide comprises at least  
2 three of the segments.

1        44. The DNA of claim 42, wherein the hybrid polypeptide comprises all six of  
2 the segments.

1        45. A DNA encoding a hybrid polypeptide the sequence of which comprises at  
2 least one of the following segments of HPV strain 16 E6 and E7 proteins:

3        AMFQDPQERPRKLPQLCTEL (SEQ ID NO:64),  
4        LLRREVVYDFAFRDLCIVYRDGNPY (SEQ ID NO:65),

5 KISEYRHYCYSLYGTTLEQQYNK (SEQ ID NO:66),  
6 TLHEYMLDLQPETTDLYSY (SEQ ID NO:67), and  
7 QAEPDRAHYNIVTF (SEQ ID NO:68),  
8 provided that the hybrid polypeptide does not comprise a sequence identical to the  
9 sequence of either full length, intact E6 or full length, intact E7 protein from HPV  
10 strain 16.

1 46. A DNA encoding a hybrid polypeptide the sequence of which comprises at  
2 least one of the following segments of HPV strain 18 E6:  
3 RRPYKLPDLCTELNTSLQDIEITCVYCKTVLELTEVFEFAFK (SEQ ID  
4 NO:152), and  
5 SVYGDTLEKLTNTGLYNLLIRCLRCQK (SEQ ID NO:153),  
6 and at least one of the following segments of HPV strain 18 E7:  
7 KATLQDIVLHLEPQNEIPV (SEQ ID NO:154),  
8 HTMLCMCKCEARI (SEQ ID NO:155), and  
9 AFQQLFLNTLSFVCPWC (SEQ ID NO:156),  
10 provided that the hybrid polypeptide does not comprise a sequence identical to the  
11 sequence of either full length, intact E6 or full length, intact E7 protein from HPV  
12 strain 18.

1 ~~Sub 237~~ 47. A DNA encoding a hybrid polypeptide the sequence of which comprises at  
2 least one of the following segments of HPV strain 16 E6:  
3 AMFQDPQERPRKLPQLCTEL (SEQ ID NO:64),  
4 LLRREYDYDFARDLCIVYRDGNPY (SEQ ID NO:65), and  
5 KISEYRHYCYSLYGTTLEQQYNK (SEQ ID NO:66);  
6 at least one of the following segments of HPV strain 16 E7:  
7 TLHEYMLDLQPETTDLYSY (SEQ ID NO:67),  
8 QAEPDRAHYNIVTF (SEQ ID NO:68), and  
9 LLMGTLGIVCRICSQKP (SEQ ID NO:69);  
10 at least one of the following segments of HPV strain 18 E6:



11 RRPYKLPDLCTELNTSLQDIEITCVYCKTVLELTEVFEFAFK (SEQ ID  
12 NO:152), and  
13 SVYGDITLEKLTNTGLYNLLIRCLRCQK (SEQ ID NO:153),  
14 and at least one of the following segments of HPV strain 18 E7:  
15 KATLQDIVLHLEPQNEIPV (SEQ ID NO:154),  
16 HTMLCMCKCEARI (SEQ ID NO:155), and  
17 AFQQLFLNTLSFVCPWC (SEQ ID NO:156).

1 48. The DNA of claim 47, wherein the hybrid polypeptide comprises at least five  
2 of the segments.

1 49. The DNA of claim 47, wherein the hybrid polypeptide comprises all eleven  
2 of the segments.

1 50. The DNA of claim 49, wherein the hybrid polypeptide further comprises a  
2 targeting signal.

1 51. The DNA of claim 50, wherein the targeting signal comprises the HLA-DR $\alpha$   
2 leader sequence (SEQ ID NO:63).

1 <sup>Sub</sup><sub>24</sub> 52. A DNA encoding a hybrid polypeptide the sequence of which comprises at  
2 least one of the following segments of HPV E6 and E7 proteins:

3 AMFQDPQERPRKLPQLCTEL (SEQ ID NO:64),  
4 LLRREVYDFAFRDLCIVYRDGNPY (SEQ ID NO:65),  
5 KISEYRHYCYSLYGTTLEQQYNK (SEQ ID NO:66),  
6 TLHEYMLDLQPETTDLYSY (SEQ ID NO:67),  
7 QAEPDRAHYNIVTF (SEQ ID NO:68),  
8 RRPYKLPDLCTELNTSLQDIEITCVYCKTVLELTEVFEFAFK  
9 (SEQ ID NO:152),  
10 SVYGDITLEKLTNTGLYNLLIRCLRCQK (SEQ ID NO:153),  
11 KATLQDIVLHLEPQNEIPV (SEQ ID NO:154),

12 HTMLCMCKCEARI (SEQ ID NO:155), and  
13 AFQQLFLNTLSFVCPWC (SEQ ID NO:156);  
14 provided that the hybrid polypeptide does not comprise a sequence identical to the  
15 sequence of either full length, intact E6 or full length, intact E7 protein from HPV  
16 strain 16 or 18.

1 53. A plasmid or viral vector comprising the nucleic acid of claim 1.

1 54. The hybrid polypeptide encoded by the nucleic acid of claim 1.

1 55. A microsphere comprising a polymeric matrix or shell and the nucleic acid of  
2 claim 1.

1 56. The microsphere of claim 55, wherein the polymeric matrix or shell consists  
2 essentially of a polymer of poly-*co*-glycolic acid (PLGA).

1 57. A therapeutic composition comprising the nucleic acid of claim 1 and a  
2 pharmaceutically acceptable carrier.

1 58. The therapeutic composition of claim 57, further including an adjuvant.

1 59. A liposome comprising the nucleic acid of claim 1.

1 60. A method of eliciting an immune response in a mammal, which method  
2 comprises administering the nucleic acid of claim 1 to the mammal.

1 61. The method of claim 60, wherein the mammal is a human.

1 62. The method of claim 61, wherein the pathogenic agent is HPV and the human  
2 suffers from, or is at risk of, a condition selected from the group consisting of exophytic  
3 condyloma, flat condyloma, cervical cancer, respiratory papilloma, conjunctival

4 papilloma, genital-tract HPV infection, cervical dysplasia, high grade squamous  
5 intraepithelial lesions, and anal HPV infection.

1 63. The method of claim 60, wherein the nucleic acid is administered directly to a  
2 mucosal tissue of the mammal.

1 64. The method of claim 63, wherein the mucosal tissue is vaginal or anal tissue.

SUB B1  
1 65. The method of claim 60, wherein the nucleic acid is administered  
2 subcutaneously or intramuscularly.

1 66. A method of eliciting an immune response in a mammal, which method  
2 comprises administering the microsphere of claim 55 to the mammal.

1 67. The nucleic acid of claim 1, wherein the first, second and third portions are  
2 portions of one or more tumor antigens expressed from a gene selected from the group  
3 consisting of the Her2/neu gene, the prostate specific antigen (PSA) gene, the melanoma  
4 antigen recognized by T cells (MART) gene, and the melanoma antigen gene (MAGE).

1 68. The nucleic acid of claim 1, wherein the first, second and third portions are  
2 portions of one or more naturally occurring proteins of one or more viruses which infect  
3 cells.

1 69. The nucleic acid of claim 1, wherein the first, second and third portions are  
2 portions of one or more naturally occurring proteins of one or more pathogenic agents  
3 selected from the group consisting of HPV, human immunodeficiency virus (HIV),  
4 herpes simplex virus (HSV), hepatitis B virus (HBV), hepatitis C virus (HCV),  
5 mycobacteria, *Helicobacter spp.*, *Chlamydia spp.*, and a parasitic eukaryote which infects  
6 cells.